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Overexpression of the Her2 protein has been found in ~30% of breast tumors, and the inhibition of Her2 expression may be an effective way to treat Her2-positive patients. Recently, the P.I. and co-workers reported identification of chemical inhibitors of Her2 transcription. The compounds that we named adamanolol and wrenchnolol inhibited Her2 transcription by disrupting the interaction of two cancer-linked nuclear proteins, ESX and Sur-2. Affinity purification revealed that wrenchnolol binds to the Sur-2 subunit of the human mediator complex by mimicking the potent activation domain of transcription factor ESX. In the third year of funding, we designed a STF1 (synthetic transcription factor), taking advantage of the ability of wrenchnolol to bind to the Sur-2 subunit and the specific DNA-binding affinity of a hairpin polyamide molecule. The hybrid compound of these two molecules activated transcription of a reporter gene *in vitro* in a promoter-dependent manner through simultaneous contacts with DNA and Sur-2. Our results indicate that wrenchnolol serves as an activation domain mimic, and that it is possible to generate a transcription factor out of completely organic components.

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Table of Contents

Introduction

Overexpression of Her2 is associated with poor overall survival and enhances malignancy and the metastatic phenotypes in breast cancers (1-5). Recently, the P.I. and co-workers reported identification of chemical inhibitors of Her2 transcription. The compounds that we named adamanolol and wrenchnolol inhibited Her2 transcription by disrupting the interaction of two cancer-linked nuclear proteins, ESX and Sur-2 (DRIP130). The drug-like molecules showed strong cytotoxicity against Her2-overexpressing breast cancer cells, but had much milder effects on breast cancer cells with no detectable levels of Her2. We also demonstrated direct interaction of wrenchnolol with Sur-2 in a cellular context, and proposed that wrenchnolol is a chemically tractable bioactive compound that is suited for further application (6, 7). The study in the third year is focused on the application of chemistry of wrenchnolol to the design of synthetic modulator of gene transcription.

Body

Naturally occurring transcription factors usually have two separable domains for activating selective genes: one for binding to specific promoters and the other for activating transcription through protein-protein interactions (8, 9). Our designed molecule **1** (STF1: synthetic transcription factor 1) comprises two functional domains (see scheme 1 in the attached paper): a hairpin polyamide molecule that binds specifically to 5'-TGACCAT sites in DNA (10) and a wrench-shaped synthetic compound (wrenchnolol) that binds to the Sur-2 protein (6, 7), a subunit of human mediator complex that links transcription activators to RNA polymerase II in human cells (11, 12). STF1 was synthesized by amide couplings of the hairpin polyamide, wrenchnolol, and a polyethylene linker. An internal pyrrole residue was chosen as an attachment site in the hairpin polyamide because previous studies had suggested that an extension at this position provides effective projection of an activation module (13, 14). The ability of STF1 to activate transcription was evaluated *in vitro* by using a reporter construct in which transcription of the reporter gene is controlled by six repeats of the hairpin-polyamide-binding sites (see Figure 1 in the attached paper). STF1 activated transcription of the reporter gene in a dose-dependent manner (lanes 2-5), whereas the control molecule **2** lacking the wrenchnolol moiety had no detectable activity (lane 6). Addition of excess amounts of a dominant negative Sur-2 protein fragment (lane 7) or immunodepletion of endogenous Sur-2 protein from nuclear extracts (lane 8) rendered the activity of STF1 undetectable,

consistent with our previous observation that wrenchnolol binds selectively to Sur-2 protein. A reporter construct with point mutations in the hairpin-polyamide-binding sites was unresponsive to STF1 (lanes 9-11), suggesting a high degree of selectivity of STF1. To verify the biochemical mechanism of the STF1-mediated gene activation, the promoter region of the reporter construct was labeled with a biotin molecule and bound to an avidin-agarose resin after incubation with nuclear extracts (see Figure 2 in the attached paper). Western blot analysis of the bound proteins showed that no detectable levels of Sur-2 or RNA polymerase II were recruited to the promoter in the absence of STF1 or in the presence of the control molecule **2**. In the presence of STF1, in contrast, Sur-2 protein and RNA polymerase II were recruited to the promoter. These results support our notion that STF1 stimulates transcription by recruiting human mediator complex to the promoter through simultaneous contacts with Sur-2 and DNA.

We are now testing wrenchnolol in animal models in collaboration with Dr Hung at MD Anderson Cancer center (Houston, TX). His laboratory has nude mice with ovarian cancer orthotopic tumor xenografts derived from SK-OV-3-ip1, a Her2-overexpressing cell line. These animals are being used to examine whether our drug lead suppresses tumor development in animal and prolong animal survival.

Key research accomplishments

1. Development of synthetic modulator of gene transcription.

Reportable outcomes

1. Asada, S., **Choi, Y.**, and Uesugi, M. (2003) A gene-expression inhibitor that targets an α-helix-mediated protein interaction. *J. Am. Chem. Soc.* **125**, 4992-4993

2. Shimogawa, H., Kwon, Y., Mao, Q., Kawazoe, Y., **Choi, Y.**, Asada, S., Kigoshi, H., and Uesugi, M. (2004) A wrench-shaped synthetic molecule that modulates a transcription factor-coactivator interaction. *J. Am. Chem. Soc.* **126**, 3461-3471

3. Kwon, Y., Arndt, H., Mao, Q., **Choi, Y.**, Kawazoe, Y., Dervan, P. B., and Uesugi, M. (2004) Small molecule transcription factor mimic. *J. Am. Chem. Soc.* **126**, 15940-15941

Conclusion

Our designed molecule **1** (STF1) comprises two functional domains: a hairpin polyamide molecule that binds specifically to 5'-TGACCAT sites in DNA and a wrench-shaped synthetic compound (wrenchnolol) that binds to the Sur-2 protein. The hybrid compound of these two molecules activates transcription of a reporter gene *in vitro* in a promoter-dependent manner through simultaneous contacts with DNA and Sur-2. Our results indicate that wrenchnolol serves as an activation domain mimic, and that it is possible to generate a transcription factor out of completely organic components.

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Appendix: attached

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COMMUNICATIONS

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Small Molecule Transcription Factor Mimic

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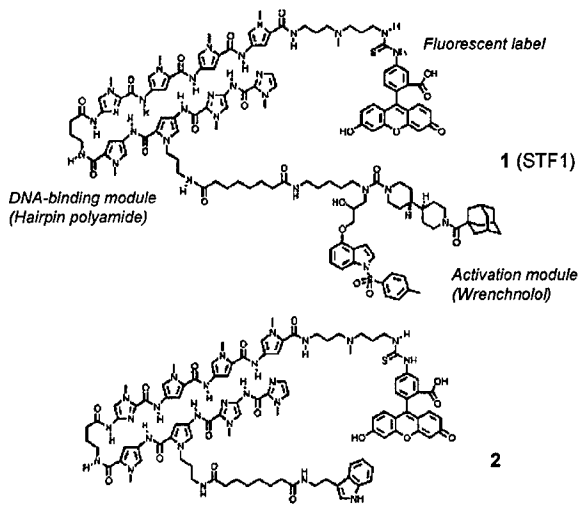
Regulation of gene expression by transcription factors touches many aspects of eukaryotic biology, and its systematic, external control by organic molecules represents a challenge in chemistry.[1,2] A number of attempts have been made to mimic transcription factors by synthetic molecules,[3−9] including those utilizing DNA-binding hairpin polyamide attached to peptide activation domains.[3,4] These studies indicate that it is possible to reproduce the gene-activation function of transcription factors by synthetic molecules. However, the presence of peptide regions in these molecules may limit their future applications. Here we report the design of a nonpeptidic small molecule that mimics a transcription factor. Naturally occurring transcription factors usually have two separable domains for activating selective genes: one for binding to specific promoters and the other for activating transcription through protein−protein interactions.[10,11] Our designed molecule **1** (STF1: *s*ynthetic *t*ranscription *f*actor 1) comprises two functional domains (Scheme 1): a hairpin polyamide molecule that binds specifically to 5′-TGACCAT sites in DNA[12] and a wrench-shaped synthetic compound (wrenchnolol) that binds to the Sur-2 protein,[13,14] a subunit of human mediator complex that links transcription activators to RNA polymerase II in human cells.[15,16]

Scheme 1



STF1 was synthesized by amide couplings of the hairpin polyamide, wrenchnolol, and a polyethylene linker. An internal pyrrole residue was chosen as an attachment site in the hairpin polyamide because previous studies had suggested that an extension at this position provides effective projection of an activation module.[4,5] The ability of STF1 to activate transcription was evaluated in vitro by using a reporter construct in which transcription of the reporter gene is controlled by six repeats of the hairpin-polyamide-binding sites (Figure 1). STF1 activated transcription of the reporter gene in a dose-dependent manner (lanes 2−5), whereas the control molecule **2** lacking the wrenchnolol moiety had no detectable activity (lane 6). Addition of excess amounts of a dominant negative Sur-2 protein fragment (lane 7) or immunodepletion of endogenous Sur-2 protein from nuclear extracts (lane 8) rendered the activity of STF1 undetectable, consistent with our previous observation that wrencholol binds selectively to Sur-2 protein. A reporter construct with point mutations in the hairpin-polyamide-binding sites was unresponsive to STF1 (lanes 9−11), suggesting a high degree of selectivity of STF1.

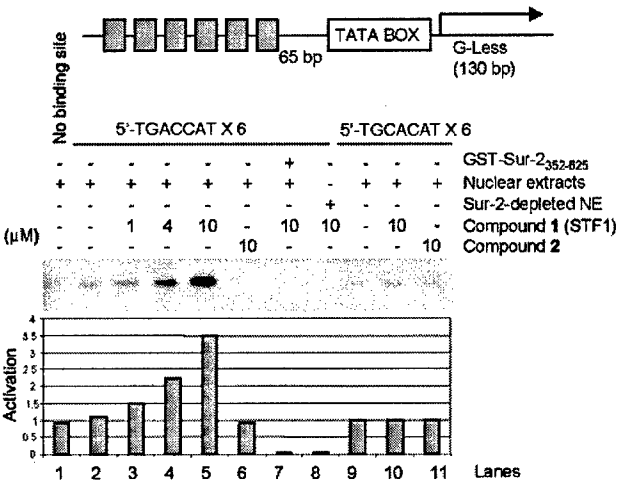


Figure 1. STF1 (**1**) activates transcription of a reporter gene in vitro. STF1 (0, 1, 4, or 10 μM) was incubated with HeLa cell nuclear extracts and a reporter DNA construct in which a G-less reporter gene is controlled by six repeats of the hairpin-polyamide-biding site (5′-TGACCAT) or those of its mutant (5′-TGCACAT). The 130-base mRNA of the reporter gene was detected by ^{32}P autoradiography and quantified by a phosphoimager. Addition of dominant negative Sur-2 protein fragment (GST-Sur-2$_{352-625}$) or immunodepletion of Sur-2 protein from nuclear extracts abolished the gene activation. It is evident that the reporter gene with mutated polyamide-binding sites is completely unresponsive to STF1 (**1**). Preparation of GST-Sur-2$_{352-625}$ and immunodepleted nuclear extracts is described in Supporting Information. A schematic diagram of the design of the reporter gene constructs is shown in the top. A gray square indicates a DNA sequence of the hairpin-polyamide-binding site.

To verify the biochemical mechanism of the STF1-mediated gene activation, the promoter region of the reporter construct was labeled with a biotin molecule and bound to an avidin−agarose resin after incubation with nuclear extracts (Figure 2). Western blot analysis of the bound proteins showed that no detectable levels of Sur-2 or RNA polymerase II were recruited to the promoter in the absence

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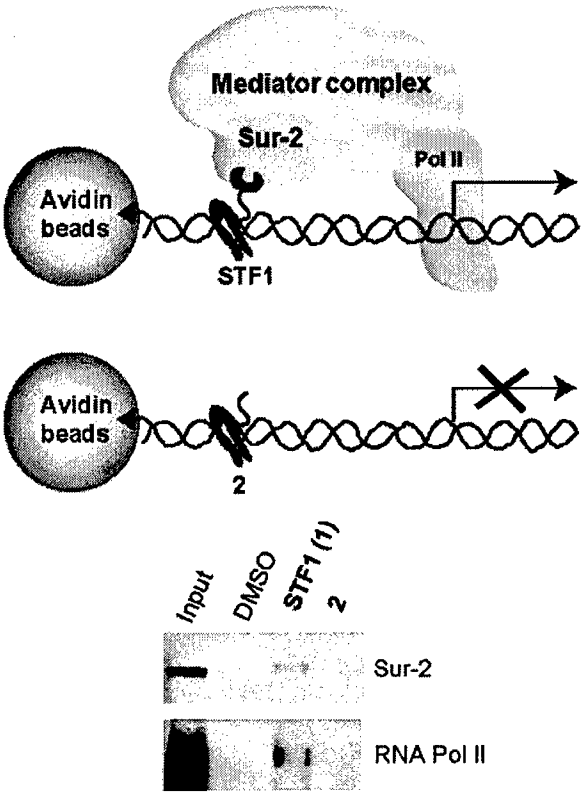

Figure 2. STF1 recruits Sur-2 and RNA polymerase II to the promoter. The promoter region of the reporter DNA construct was labeled by a biotin molecule and incubated with HeLa cell nuclear extracts in the presence of **1**, **2**, or solvent (DMSO) alone. The biotinylated DNA was recovered by using Neutravidin agarose, and the bound proteins were analyzed by Western blots with antibodies against Sur-2 and RNA polymerase II.

of STF1 or in the presence of the control molecule **2**. In the presence of STF1, in contrast, Sur-2 protein and RNA polymerase II were recruited to the promoter. These results support our notion that STF1 stimulates transcription by recruiting human mediator complex to the promoter through simultaneous contacts with Sur-2 and DNA.

Our results indicate that it is possible to generate a transcription factor out of nonpeptidic components.[17] The fluorescein moiety of STF1 permitted evaluation of its cell permeability.[12] Unfortunately, STF1 had limited cell permeability, even though the hairpin-polyamide–FITC conjugate and the wrenchnolol molecule are cell permeable as separate compounds.[1,12,14,18] By decreasing the size of the molecule and optimizing its physical properties, it may be possible to increase its cell permeability for biological studies.

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Supporting Information Available: Detailed experimental procedures. This material is available free of charge via the Internet at http://pub.acs.org.

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